REMARKS/ARGUMENTS

In response to the Office Action of June 8, 2005, Applicants request re-examination and reconsideration of this application for patent pursuant to 35 U.S.C. 132.

Claim Status/Support for Amendments

Claims 2 and 5 have been amended. New claims 6 and 7 have been added. Claims 1, 3 and 4 are withdrawn from consideration. It is understood that claims 1, 3 and 4, drawn to the non-elected invention, will remain pending, albeit withdrawn from prosecution on the merits at this time.

Claims 2 and 5-7 are currently under examination. Claims 1-7 remain pending in the instant application.

No new matter has been added by the amendments to the specification made herein.

The brief description of Figures 1-3, at page 10, has been amended to describe each panel of the figures separately, i.e. Figures 1A-1C. Each panel of Figures 1-3 was described in the specification as originally filed at pages 11-12.

The paragraph at page 20 was amended to add sequence identification numbers to the peptides disclosed therein.

No new matter has been added by the amendments to the claims made herein.

Claims 2 and 5 were amended to clarify that administration of an ABBOS peptide can alleviate and/or reduce the symptoms of primary Sjögren's Syndrome, such as sialoadenitis and dacryadenitis, in mammals exhibiting said symptoms. Pages 16-23 of the instant specification as originally filed describe experiments which evidence the veracity of this statement.

No new matter has been added by the addition of new claims 6 and 7. New claims 6 and 7 identify the ABBOS peptide as SEQ ID NO:2. The amino acid sequence of the ABBOS peptide is disclosed at page 20, line 7 of the instant specification as originally filed and is identified as SEQ ID NO:2 in the Sequence Listing filed herewith.

Drawings

The drawings, as originally presented, stand objected to under 37 CFR 1.83(a) because they allegedly fail to show the histological details and banding patterns as described in the specification. Specifically, the Examiner asserts that Figures 1-3 contain subsections which are not listed in the Brief Description of the Figures.

The Brief Description of the Drawings section of the instant specification at page 10 has been amended herein to identify and describe each panel of Figures 1-3 separately, i.e. Figures 1A-1C.

No new matter has been added with these changes to the figure descriptions as each panel of Figures 1-3 was described separately at pages 11-12 of the instant specification as originally filed.

When reviewing the figures as originally filed, Applicants noted several formatting and typographical errors. Thus, Applicants submit herewith a set of corrected drawings to replace the drawings as originally filed. The corrected drawings are labeled "Replacement Sheet" in the page header according to 37 CFR 1.84(c). A set of annotated drawings showing changes made is also filed herewith.

Applicants respectfully submit that the drawings are now in compliance with all of the drawing rules and thus, respectfully request that the objection to the drawings now be withdrawn.

Sequence Compliance

Applicants have reviewed the entire specification, including the figures and the claims, for sequence disclosures. Two peptide sequences were found to be disclosed at page 20 and were identified as SEQ ID NOS:1 and 2, respectively.

Applicants herein provide an electronic computer-readable form (diskette) containing a Sequence Listing identifying the two peptides. Additionally, Applicants herein provide a paper copy of the Sequence Listing as found on the diskette filed herewith. The

computer-readable form of the Sequence Listing is identical to the paper copy of the Sequence Listing. The amino acid sequences of SEQ ID NOS:1 and 2 were disclosed in the specification as originally filed at page 20, lines 2-13; thus, no new matter has been added by the addition of sequence identification numbers.

Applicants respectfully submit that the instant application is now in compliance with 37 CFR 1.821-1.825 (sequence rules).

Rejection under 35 USC 112, first paragraph/Written Description

Claims 2 and 5, as presented on April 25, 2005, stand rejected under 35 USC 112, first paragraph, as allegedly failing to comply with the written description requirement.

The Examiner asserts that the claims contain subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time that the application was filed, had possession of the claimed invention.

The Examiner alleges that the claims are drawn to any high affinity mimicry peptide targeting ICA69-specific T cells from any species. And further, the claims encompass a genus of mimicry peptides defined solely by the fact that they have a high affinity for ICA69-specific T cells.

Applicants do not acquiesce to the Examiner's assertions,

however, in the interest of compact, efficient prosecution, Applicants have amended the claims to specifically recite the ABBOS peptide (SEQ ID NO:2).

Thus, Applicants respectfully request that this rejection under 35 USC 112, first paragraph (written description) be withdrawn.

Rejection under 35 USC 112, first paragraph/Enablement

Claims 2 and 5, as presented on April 25, 2005, stand rejected under 35 USC 112, first paragraph, because the specification, while being enabling for an immunotherapeutic process for treating a NOD mouse suffering from primary Sjögren's Syndrome with an ABBOS high affinity mimicry peptide targeting ICA69-specific T cells in a manner effective to induce tolerance to ICA69, whereby the symptoms characteristic of primary Sjögren's Syndrome are treated, such as a reversal of sialoadenitis and dacryladenitis, does not reasonably provide enablement for an immunotherapeutic process for treating any individual suffering from primary Sjögren's Syndrome with any high affinity mimicry peptide targeting ICA69-specific T cells in a manner effective to induce tolerance to a relevant ICA69 epitope, whereby the symptoms characteristic of primary Sjögren's Syndrome are treated, such as a reversal of sialoadenitis and dacryladenitis. The specification does not enable any person

skilled in the art to which it pertains, or with which it is mostly nearly connected, to practice the invention commensurate in scope with these claims.

Applicants respectfully disagree with the Examiner's determination.

Applicants draw the Examiner's attention to the fact that the claims have been amended to recite administration of the ABBOS peptide to a mammal exhibiting the symptoms of primary Sjögren's Syndrome and are no longer drawn to treatment of any individual with any high affinity mimicry peptide.

The MPEP provides guidelines that indicate as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 USC 112, first paragraph is satisfied (see MPEP 2164.01(b)).

Furthermore, if the art is such that a particular model is recognized as correlating to a specific condition, then it should be accepted as correlating (see MPEP 2164.02).

The instant specification discloses an example of a process for alleviating and/or reversing the symptoms of primary Sjögren's Syndrome in a NOD mouse by administering an ABBOS peptide to the NOD mouse which targets ICA-specific T-cells in a manner effective to induce tolerance to ICA69 in the NOD mouse (see the instant

specification as originally filed at pages 14-23).

Applicants respectfully assert that the murine model of Sjögren's Syndrome is recognized as correlating to the human condition of Sjögren's Syndrome, see, for example, Hayashi and Jabs et al.

Autoimmune disease-prone mice such as NZB, NZB/WF1, MRL/1pr, SL/Ni, and NOD (non-obese diabetic) have been used as animal models for the investigation of Sjögren's Syndrome in humans (see attached abstract of Hayashi, Y. Nippon Rinsho. 53(10):2383-2388 1995; reference 1).

Autoimmune mice provide models for the human disorder Sjögren's Syndrome and a mechanism for better understanding the immunopathogenesis of autoimmune lacrimal gland disease (see attached abstract of Jabs et al. Adv Exp Med Biol. 350:623-630 1994; reference 2).

The murine model is especially acceptable with regard to Sjögren's Syndrome since murine ICA69 has sequence identity with the human protein in the Tep69 region (see Karges et al. Diabetes 46:1548-1556 1997, page 1552, first paragraph of section entitled "Discussion").

Additionally, much of the art actually encourages extrapolation of data obtained from investigations in NOD mice to humans, see, for example, Atkinson et al. (Nature Medicine

5(6):601-604 1999; reference 3). Atkinson et al. states the following at page 601, first column on the left:

"Today, when a candidate autoantigen undergoes evaluation, the effect of a cytokine is tested, or a preventive intervention is assessed, NOD mice are often considered as good as it gets, short of a study in humans-so much so that other animal models are not always tested **nor are important distinctions with the NOD model considered before extrapolations to humans are made.**" (emphasis added by Applicants).

Thus, Applicants respectfully submit that one of ordinary skill in the art when reviewing the experiments disclosed in the instant specification involving NOD mice, given the level of knowledge and skill in the art regarding NOD mice as models for autoimmune disease, would accept that the data obtained can be applied to autoimmune disease in mammals, such as humans.

Accordingly, Applicants respectfully request that this rejection under 35 USC 112, first paragraph now be withdrawn.

Rejection under 35 USC 103(a)

Claims 2 and 5, as presented on April 25, 2005, stand rejected under 35 USC 103(a) as allegedly being unpatentable over Karges et al. (Diabetes 46:1548-1556 1997) in view of Humphreys-Behr (Adv Dent Res 10(1):73-75 1996).

According to the Examiner, Karges et al. is deemed to provide guidance on treatment of NOD mice with the ABBOS mimicry high-affinity peptide, in order to induce T-cell tolerance to ICA69. Further, Karges et al. teaches that administration of the ABBOS mimicry peptide reduced diabetes incidence in NOD mice and was able to induce cross-tolerance to the Tep69 epitope of ICA69 autoantigen. The Examiner notes that Karges et al. does not treat the NOD diabetic mice that have primary Sjögren's Syndrome.

According to the Examiner, Humpreys-Behr supplements the guidance of Karges et al. by teaching that the diabetic NOD mouse model also undergoes a corresponding loss in exocrine gland function related to infiltrates symptomatic of the pathophysiology of primary Sjögren's Syndrome.

Based on the guidance provided by Karges et al. on the method of treating diabetes in NOD mice with the ABBOS mimicry high-affinity peptide by inducing tolerance of the mouse's ICA69-specific T cells to ICA69, and the guidance of Humpreys-Behr that some diabetic NOD mice develop primary Sjögren's Syndrome, the

Examiner asserts that it would be *prima facie* obvious to the person of ordinary skill in the art at the time of the invention that treatment of diabetic NOD mice with the ABBOS mimicry high-affinity peptide that induced tolerance in ICA69 specific T cell to ICA69 would also treat any other disease caused by the activity of ICA69 specific T cells, such as primary Sjögren's Syndrome in the same mouse. A practitioner in the art would be motivated to treat NOD mice with diabetes and primary Sjögren's Syndrome with the ABBOS peptide in order to induce tolerance of the mouse's ICA69 specific T cells to ICA69 and thus to treat the diabetes. The person of ordinary skill in the art would have a reasonable expectation of success because the method of Karges et al. treats diabetes in the mouse by inducing tolerance in ICA69 specific T cells and therefore any other diseases caused by these ICA69 specific T cells would also be treated by the induction of tolerance.

Applicants respectfully disagree with the Examiner's determination that the claimed subject matter is obvious.

In order for an Examiner to establish a *prima facie* case of obviousness, three basic criteria must be met (MPEP 2142). First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable

expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations.

Apparently, the Examiner believes that treatment of diabetic NOD mice with the ABBOS peptide will also treat any other disease caused by the activity of ICA69 specific T Cells, in particular, primary Sjögren's Syndrome in the same treated NOD mice.

Mice of the NOD mouse strain NOD.B10.H2^{b} do not express the MHC I-A^{g7} antigen deemed essential for the development of insulitis and diabetes (in NOD mice); thus in the search for a model of primary Sjögren's Syndrome exocrine gland function was studied in this NOD strain. It was found that these mice, NOD.B10.H2^{b}, exhibit the exocrine gland lymphocytic infiltration typical of Sjögren's Syndrome but without the insulitis and diabetes. Thus, autoimmune diabetes and Sjögren's Syndrome in NOD mice can be separated genetically and were recognized as genetically separate at the time of the invention. See the attached article of Robinson et al. Arthritis & Rheumatism 41(1):150-156 1998, reference 4, for a discussion of Sjögren's Syndrome and NOD.B10.H2^{b} mice.

Furthermore, prior to the instant invention, the ICA69 autoantigen was not known to be involved in primary Sjögren's Syndrome, i.e. primary Sjögren's Syndrome was not known to be caused by the activity of ICA69 specific T cells.

Neither of the cited references suggests any connection between the ICA69 autoantigen and primary Sjögren's Syndrome.

Karges et al. disclose that ICA69 is a target autoantigen in autoimmune diabetes, but does not mention or suggest involvement of ICA69 in primary Sjögren's Syndrome. Karges et al. also disclose that antigenic mimicry exists between ICA69 and BSA. Cross-tolerance, as disclosed by Karges et al., indicates that the T cell pools recruited by ICA69 and BSA are similar. Karges et al. is silent with regard to any connection between this antigenic mimicry (ICA69 and BSA) and primary Sjögren's Syndrome.

Humpreys-Behr discusses the symptoms of Sjögren's Syndrome only as secondary to other autoimmune diseases and makes no mention of the autoantigen ICA69.

Why would one of ordinary skill in the art at the time that the invention was made expect that since induction of tolerance to ICA69 was successful in treatment of diabetes in NOD mice, induction of tolerance to ICA69 would also be successful in treatment of primary Sjögren's Syndrome in NOD mice considering that at the time the ICA69 autoantigen was not known to be involved in primary Sjögren's Syndrome and further that diabetes and primary Sjögren's Syndrome were considered genetically separate?

Accordingly, neither the cited references nor the knowledge available to those of skill in the art at the time of the invention

suggest any connection between the autoantigen ICA69 and primary Sjögren's Syndrome. Without knowledge of a connection between ICA69 and primary Sjögren's Syndrome, one of ordinary skill in the art would not be able to ascertain any advantages for modifying the teachings of Karges et al. and Humpreys-Behr to treat primary Sjögren's Syndrome in NOD mice by the induction of tolerance to ICA69 and therefore, would not have any motivation for making these modifications.

Furthermore, Applicants respectfully submit that the Examiner's conclusion of obviousness is based upon impermissible hindsight since the Examiner used information gleaned only from Applicants' disclosure; i.e. the involvement of the ICA69 autoantigen in primary Sjögren's Syndrome (see MPEP 2145 X. A. for a discussion of "impermissible hindsight").

The Examiner must also show that one of ordinary skill in the art would have a reasonable expectation of success when modifying the reference or combining reference teachings.

Since neither the cited references nor the knowledge available to those of skill in the art at the time of the invention teach any connection between the autoantigen ICA69 and primary Sjögren's Syndrome; one of ordinary skill in the art would have no reason to expect success in treatment of primary Sjögren's Syndrome by induction of tolerance to ICA69 because the ICA69 autoantigen was

not known to be part of the pathogenesis of primary Sjögren's Syndrome.

It has been established that the cited references (Karges et al. and Humpreys-Behr)do not teach or suggest all of the limitations of claims 2 and 5, since neither reference connects the pathogenesis of primary Sjögren's Syndrome to the ICA69 autoantigen.

Thus, Applicants respectfully submit that the Examiner has failed to satisfy all the criteria necessary to establish a proper rejection of claims under 35 USC 103(a); 1) suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art to modify the references or to combine teachings; 2) reasonable expectation of success and 3) the reference or references when combined must teach or suggest all of the claim limitations.

In light of all of the above remarks, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness and further contend that a practitioner of ordinary skill in the art, having the cited references (Karges et al. and Humpreys-Behr) in front of him/her would not have the information and motivation necessary to arrive at Applicants' invention.

Thus, it is respectfully submitted that the combination of the teachings of Karges et al. and Humpreys-Behr fails to

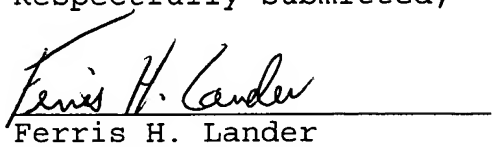
reasonable teach or suggest to one of ordinary skill in the art the elements of Applicants' processes as specifically set forth in claims 2 and 5-7 as presented herein.

Accordingly, Applicants respectfully submit that the claimed processes distinguish over the prior art and respectfully request that this rejection of claims 2 and 5 under 35 USC 103(a) now be withdrawn.

CONCLUSION

In light of the foregoing remarks, amendments to the specification and amendments to the claims, it is respectfully submitted that the Examiner will now find the claims of the application allowable. Favorable reconsideration of the application is courteously requested.

Respectfully submitted,



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2560.001 Examiner copy reference #1

1: Nippon Rinsho. 1995 Oct;53(10):2383-8.

[Investigations of various animal models for Sjogren's syndrome]

[Article in Japanese]

Hayashi Y.

Department of Pathology, Tokushima University School of Dentistry.

Several animal models for studying immune-mediated sialadenitis include autoimmune-prone mice which develop lesions spontaneously, and non-autoimmune-prone mice in which the lesions can be induced by various experimental manipulations. Autoimmune disease-prone mice such as NZB, NZB/WF1, MRL/lpr, SL/Ni, and NOD (non-obese diabetic) have been used as animal models for the investigation of Sjogren's syndrome in humans. We have previously demonstrated murine experimental systems in which autoimmune sialadenitis was induced in several strains of thymectomized mice, and developed spontaneously in certain strains of aged mice with H-2 restriction. We have recently established an animal model for primary Sjogren's syndrome in NFS/sld mutant mice bearing an autosomal recessive gene with sublingual gland differentiation arrest. A significantly higher incidence of autoimmune lesions in the salivary and lacrimal gland was found in female mice, and the anti-salivary duct autoantibodies were detected in sera from mice with autoimmune lesions. A preferential use of TCRV beta gene was detected in autoimmune lesions from the onset of disease, suggesting that TCR-based immunotherapy is possible.

Publication Types:
- Review
- Review, Tutorial

PMID: 8531342 [PubMed - indexed for MEDLINE]

Aug 16 2005 14:03:09

2560.001

Examiner copy reference #2

1: Adv Exp Med Biol. 1994;350:623-30.

Murine models of Sjogren's syndrome.

Jabs DA, Prendergast RA.

Wilmer Ophthalmological Institute, Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Autoimmune MRL/lpr, MRL/+, and NZB/W mice all develop lacrimal gland inflammatory lesions, which consist of focal mononuclear inflammatory cell infiltrates. Each strain has a different immunocytochemical profile, which appears to be related to the underlying immunologic defects present in that mouse. The appearance of these lesions parallels the evolution of the systemic autoimmune disease. The lesions are dynamic over time with the early appearance of CD4+ T cells (helper T cells) for each strain. Subsequently, there is an accumulation of B cells over time in MRL/+ and NZB/W mice. In the two more rapidly evolving mouse models, MRL/lpr and NZB/W, there is a progressive decline in the percentage of CD8+ cells. Conversely, in the slowly evolving MRL/+ lacrimal gland lesions, there is a persistent and unchanging percentage of CD8+ T cells (suppressor/cytotoxic T cells). Autoimmune mice provide models for the human disorder Sjogren's syndrome and a mechanism for better understanding the immunopathogenesis of autoimmune lacrimal gland disease.

Publication Types:
- Review
- Review, Tutorial

PMID: 8030545 [PubMed - indexed for MEDLINE]

Aug 16 2005 14:03:09

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Examiner copy

reference # 3

Of the two well-known rodent models of human type 1 diabetes (the non-obese diabetic mouse and the BioBreeding rat) the mouse has become the model of choice. Here the authors re-examine the value of this mouse model as a tool for understanding human diabetes and for testing potential therapies, reviewing both strengths and weaknesses.

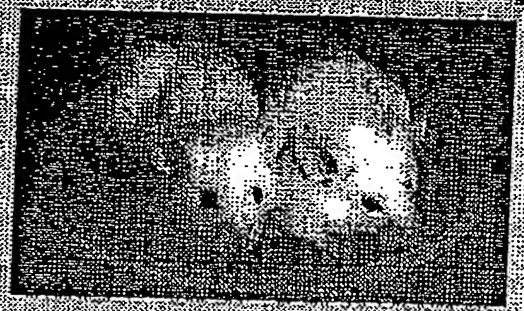
The NOD mouse model of type 1 diabetes: As good as it gets?

MARK A. ATKINSON[1] & EDWARD H. LEITER[2]

Diabetes mellitus in humans is a genetically and clinically heterogeneous group of glucose intolerance syndromes. Type 2 diabetes (also called non-insulin-dependent diabetes mellitus) is the more prevalent clinical form, in which obesity associated with progressively more severe insulin resistance are common predictors of the prediabetic state. Type 1 diabetes (also called insulin-dependent diabetes mellitus, or juvenile diabetes), in contrast, usually has an autoimmune T cell-mediated etiology in which the prediabetic state is characterized by development of autoantibodies against certain proteins expressed by β cells, including insulin. Two rodent models that spontaneously develop type 1 diabetes, the NOD (non-obese diabetic) mouse and the *BB* (BioBreeding) rat, have allowed detailed exploration of the dysregulated communication between cells of the innate and acquired immune system that underlie the generation and release of pancreatic β cell-reactive T cells.

In the 19 years since the first report of the NOD mouse, this small rodent has eclipsed its 'bigger brother', the BB rat, as the favored model for investigations into the etiopathogenesis of autoimmune, T cell-mediated type 1 diabetes in humans. The reasons for the preferred popularity of the mouse model include a better-defined genome, more monoclonal reagents for the analysis of immune system components and considerably lower maintenance costs. Today, when a candidate autoantigen undergoes evaluation, the effect of a cytokine is tested or a preventative intervention is assessed, NOD mice are often considered 'as good as it gets', short of a study in humans—so much so that other animal models are not always tested nor are important distinctions with the NOD model considered before extrapolations to humans are made.

Indeed, the introduction of NOD mice to diabetes research infused a large sense of optimism, with immunologists initially assuming that the NOD mouse would be a murine 'Rosetta stone' for quickly unraveling the secrets of the etiopathogenesis of type 1 diabetes in humans. This belief found early support through the observation that, as in humans, the major histocompatibility complex (MHC) of the NOD mouse (designated $H2^{g7}$) contributed the main component of susceptibility, and that the MHC class II I-Aβ chain showed the same 'diabetogenic' amino-acid substitution found in the human *DQ*0302* allele associated with high risk for development of type 1 diabetes (a non-aspartic-acid substitution at residue 57 in the β-chain). Rapid cloning of the effector T cell, sequencing of its T-cell receptor (TCR) genes and identification of cognate peptide(s) were predicted to follow. From there, identification of the target β-cell autoantigen and development of blocking peptides or tolerogenic regimens were to be relatively simple matters. Furthermore, it was assumed that identification of non-MHC diabetogenic loci in the NOD mouse would allow for rapid identification of human homologs, thereby allowing accurate prediction of children at high genetic risk for developing type 1 diabetes. Finally, if the target autoantigen in mouse and human β cells were the same, the prevention of type 1 diabetes in the NOD mouse would rapidly be followed by comparable immunologic prediction and, hopefully, the eradication of this disease in humans.



Origin of NOD mice. The NOD mouse (depicted at left) arose from outbred Swiss mice in the course of a selection experiment in Japan in which brother x sister matings were being used to produce a strain in which all mice developed cataracts. At an early generation of inbreeding, mice without cataracts, but with elevated fasting blood glucose (a clinical sign of pre-diabetes) were noted. These were selectively bred in the hope of creating a mouse model for spontaneous diabetes development. The end result of this selection was the inbred NON strain depicted on the right, a model of pre-type 2 diabetes. The NOD strain (left) derived from a co-selected control line exhibiting normal fasting blood glucose levels, the first case of spontaneous autoimmune type 1 diabetes occurred unexpectedly in that 'normoglycemic' control line at the twentieth generation of sibling mating. When these two related strains are intercrossed to elucidate the chromosomal locations of diabetes susceptibility genes, homozygous expression of the NOD MHC appears essential for disease development, in contradistinction to the genetics of human type 1 diabetes, in which heterozygosity for MHC genes is common. When the chromosomal locations of non-MHC genes are identified, the NOD parental strain, as expected, contributes most of the genetic susceptibility. However, some susceptibility also derives from the NON strain genome. This illustrates the complexity of diabetes genetics in an outbred human population, wherein overlapping susceptibilities for type 1 and type 2 diabetes may be inherited in some individuals.

Our understanding of the pathogenic mechanisms underlying type 1 diabetes development in NOD mice is now quite advanced[1]. However, this understanding has been accompanied by the realization that when this mouse is used as a surrogate for humans, genus-specific differences that restrict their interpretation are unavoidable. In addition to certain NOD strain-

COMMENTARY

Table The 'A-to-Z' of diabetes prevention in the NOD mouse. Therapies include those that either suppress T-cell functions or stimulate the immune system to achieve a more normal immunoregulatory communication between antigen-presenting cells and T cells.

Androgen
Anesthesia
Azathioprine
Anti-B7-1
Bacille Calmette Gue'rin (BCG)
Baculofin
β-1,6;1,3-D-glucan
Anti-β 7 integrin
Blocking peptide of MHC class II
Bone marrow transplantation
Castration
Anti-CD3
Anti-CD4
Anti-CD8
Anti-CD28
Cholera toxin-B subunit
Cold exposure
Anti-complement receptor
Complete Freund's adjuvant
Anti-CTLA-4
Cyclosporin
Cyclosporin A
Dapsome (4,4'-diaminodiphenyl sulfone)
Deflazacort
Dendritic cells from pancreatic lymph node
Deoxysperguolin
Diazoxide
1,25 dihydroxyl Vitamin D3
Elevated temperature
Encephalomyocarditis virus (ECMV)
Escherichia coli extract
Essential fatty acid-deficient diets
FK506
Galium nitrate
Glucose (neonatal)
Glutamic acid decarboxylase
-intraperitoneal, intrathymic, intravenous, oral
Glutamic acid decarboxylase peptides
-intraperitoneal, intrathymic, intravenous, oral
Gonadectomy
Heat shock protein 65
Heat shock protein peptide (p277)
Anti-ICAM-1
Immobilization
Immunoglobulin (IgG2a)
Anti-integrin alpha 4
Inomide
Insulin
-intraperitoneal, oral, subcutaneous, nasal
Insulin B chain/B chain amino acids 9–23
-intraperitoneal, oral, subcutaneous, nasal
Insulin–metabolically inactive
Insulin-like growth factor I
Interferon-α
Anti-interferon-γ
Interferon-γ receptor
Interleukin-1
Interleukin-1 receptor
Interleukin-2
Interleukin-2 receptor fusion toxin (DAB480-IL-2)
Interleukin-3
Interleukin-4
Interleukin-10
Interleukin-12 antagonist
Islet cells-intrathymic
Lactate dehydrogenase virus (LDH)
Lactobacilus casei
Lazaroid
Linomide
Lithium chloride
Anti-LFA-1
Anti-L-selectin
Lymphocyte chorlomeningitis virus (LCMV)
Anti-lymphocyte serum/lymphotoxin
Lymphocyte vaccination
LZ8
MDL 29311
Melatonin
Anti-MHC class I
Anti-MHC class II
Mixed allogeneic chimerism
Monosodium glutamate
Murine hepatitis virus (MHV)
Mycobacterium
Natural antibodies
Nicotinamide
Nutramigen
OK432
Overcrowding
Pancreatectomy
Pentoxifylline
Pertussigen
Poly [I:C]
Pregestimil diet
Probucol
Prolactin
Rampamycin
Reg protein
Rolipram
Saline (repeated injection)
Semi-purified diet (AIN-76)
Silica
Sodium fusidate
Somatostatin
Non-specific pathogen free conditions
Streptococcal enterotoxins (SEA)
Superantigens
Superoxide dismutase-desferrioxamine
TGF-β
Anti-T-cell receptor
Anti-thy-1
Thymectomy (neonatal)
T-lymphocyte clones
Tolbutamide
Troglitazone
Tumor necrosis factor-α
Tumor necrosis factor-β
Vitamin E
Anti-VLA-4

specific characteristics that distinguish these mice from humans at risk for type 1 diabetes (such as deafness or the absence of C5 complement), important genus-specific features distinguish the murine diabetes as well (such as resistance to ketoacidosis or the absence of the murine homolog of HLA-DR molecules on antigen-presenting cells). Investigators have not always considered that because these mice are so highly inbred, they must be viewed as a single 'case study' in humans. Indeed, the combination of NOD strain-specific features as well as inherent differences between genera may explain why identification of non-MHC diabetogenic loci in mice have not generally been direct guide posts for the identification of homologous loci in outbred humans at risk for type 1 diabetes. As an example, the non-MHC locus (*IDDM2*, chromosome 11) that contributes to increased sibling risk in human studies is associated with a variable-number tandem repeat controlling expression of the closely-linked insulin gene. A susceptibility-conferring homolog has not yet been identified in the NOD mouse, most likely because the mouse genome, unlike the human genome, contains two unlinked insulin genes, both of which are expressed. Nevertheless, certain immunogenetic and immunopathogenic aspects of type 1 diabetes in this mouse 'case study', particularly the main pathogenic contributions made by MHC genes (*idd1* in NOD mice and *IDDM1* in humans), clearly justify thorough investigation into why MHC-associated deficiencies in immune function allow development of an autoreactive T-cell repertoire.

The etiology of type 1 diabetes in this model is both complex and multifactorial[1–3]. Both $CD4^+$ and $CD8^+$ T cells constitute the effector arm, with underlying functional defects in bone marrow-derived antigen-presenting cells (APCs), including macrophages, dendritic cells and B lymphocytes, shown to be essential components in selection/activation of the autoimmune repertoire. Many $CD4^+$ and $CD8^+$ T-cell lines and clones with diabetogenic potencies against a variety of identified and unidentified antigens have been established from both islets and spleen[2]. If there is a single TCR clonotype distinguishing the 'primordial' diabetogenic T cell, its primacy has not yet been demonstrated. Destruction of β cells apparently entails both necrotic and apoptotic events in response to invasion of the islets by leukocytes (insulitis)[3]. There are large numbers of leukocytes in the insulitic infiltrates of NOD mice, almost suggesting lymph node formation around islets. Insulitis in a human acute-onset diabetic is very different from that in NOD islets (Fig.). One of the strain-specific peculiarities of NOD mice is the accumulation of many T cells in peripheral lymphoid organs, pancreas and submandibular salivary glands. This T-lymphocyte accumulation possibly reflects low IL-2 levels and the resistance of thymocytes and peripheral T cells to the induction of apoptosis.

Although they are important in improving our understanding of the cause(s) and pathogenesis of this disease, these immunologic features are also vital for this model to serve as a tool in identifying potential therapeutic modalities for the prevention of human type 1 diabetes. As of early 1999, more than 125 individual methods reporting the prevention or delay of

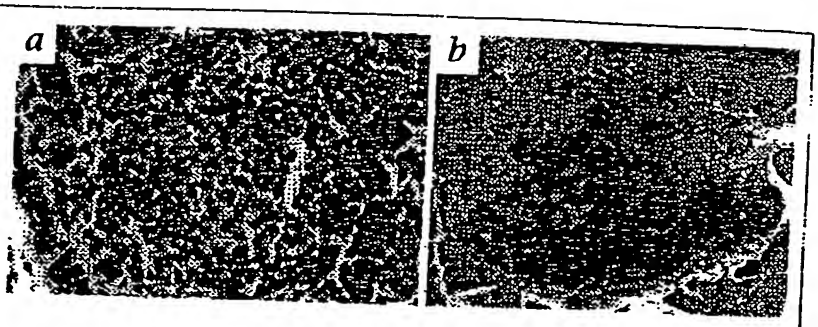


Fig. 1. Insulitis in humans and NOD mouse pancreas. *a*, Pancreas section with heavy leukocyte infiltration (insulitis) in a human islet. The donor died a few days after acute onset of type 1 diabetes. (photo courtesy of Massimo and Trucco, Department of Pediatrics, University of Pittsburgh). Hematoxylin and eosin stain. *b*, Pancreas of a prediabetic 12-week-old NOD/Lt mouse, showing the unusually heavy accumulation of leukocytes adjacent to and infiltrating the islets. The pancreatic β cells with in the islet have been stained purple with aldehyde fuchsin.

type 1 diabetes in NOD mice have been identified (Table). These interventions can be grouped into two general categories: treatments that actually suppress T-cell function and treatments that modulate immune communication, often by actually stimulating certain immune functions. Although this list is long, we limited reference to studies monitoring spontaneous type 1 diabetes and excluded reports whose practical relevance to human disease is unclear. (For example, introduction of genetically-disrupted genes whose normal counterparts are required for (auto)antigen presentation, for T-cell effector functions, or for rearrangement of functional T-cell receptors.) The ease by which immunomodulation diverts the immune system in these mice is best understood by considering the effect of their exposure to extrinsic microbial pathogens. The inbreeding of NOD mice has genetically fixed a number of immunodeficiencies that, in aggregate, are reflected by impaired communication between APC and T cells. NOD macrophages have an impaired ability to activate regulatory T cells in an autologous mixed lymphocyte reaction. Comparable impairment in dendritic cell function has been seen in patients with recent-onset type 1 diabetes[4]. The NOD immunodeficiences are partially correctable in a natural environment, in which a full range of microbial and viral antigens would be encountered. It is only when NOD mice are maintained in stringent specific pathogen-free conditions that full disease penetrance of the underlying genetic type 1 diabetes susceptibility will be seen in both sexes. Thus, the NOD model is one in which paradoxical immunostimulation effected by a variety of treatments ameliorates the weak communication between the innate and adaptive immune system components and thereby restores more normal control over autoreactive T cells. Unfortunately, in a genetically heterogeneous human population containing individuals at high risk for type 1 diabetes development, there is little evidence that many of them would have a comparable set of immune deficiencies that prove as malleable. At the same time, the observation that 'cleaning up' the extrinsic environment of the NOD mouse sets the stage for activation of autoimmune T cells raises the question of whether a hypersanitized rearing environment for human infants might predispose children with autoimmune-permissive HLA haplotypes to higher risk for eventual penetrance of autoimmune diseases.

In NOD mice, type 1 diabetes development is well-choreographed when all the relevant environmental factors (pathogen status, diet and so on) are held constant. Specific 'time windows' can be defined in which an immunomodulator can be either protective or destructive. In contrast, the natural history of type 1 diabetes in humans is such that the age of disease onset is extremely broad; symptoms occur at any time from the first years of life to well beyond 50 years of age. Although it is a potential limitation in comparing the natural history of type 1 diabetes in NOD mice with humans, this factor can be turned into a strength through proper matching of therapeutic agents to the appropriate time for human intervention. Intervention studies in NOD mice can be designed in which therapeutic regimens are initiated at birth, at a presymptomatic stage before the occurrence of insulitis (that is, less than three weeks postpartum); before the onset of symptomatic disease (that is, four to eight weeks postpartum) at a time when considerable numbers of β cells are still intact; or at the diagnosis of type 1 diabetes, when β-cell damage has accumulated to the extent of overt hyperglycemia. However, studies analyzing therapeutic agents aimed at preventing type 1 diabetes in NOD mice must be carefully assessed for their functional as well as their practical applicability to therapeutic intervention in human disease. This has not always been considered. For example, agents used in NOD mice from birth (a time without β-cell destruction) may not be applicable to treatment of humans identified immediately before the onset of type 1 diabetes (when substantial β-cell destruction has occurred).

It is clear that the genus-unique and strain-specific aspects of diabetogenesis in NOD mice must be fully understood and appreciated if we are to know which therapeutic protocols are reasonable to extrapolate to humans and which are not. In addition, intervention protocols effective in preventing type 1 diabetes in NOD mice should be studied in as many animal models as are available. The rationale for implementing insulin prophylaxis therapy to prevent type 1 diabetes in humans was based on the observation that insulin treatment of both prediabetic NOD mice and BB rats retarded onset and reduced disease frequency. However, one may question whether another candidate β-cell autoantigen, glutamic acid decarboxylase (GAD), will provide similarly promising results. Isoforms of this enzyme are relatively easy to detect in human and rat β cells. In contrast, GAD protein is present at considerably lower concentrations in NOD islets and what little GAD67 isoform can be detected may not all derive from β cells. Nevertheless, NOD mice can be deviated from diabetes by early treatment with recombinant GAD protein or peptides. In contrast, GAD autoimmunity does not seem to be a factor in BB rats, emphasizing the point that many models should be evaluated before extrapolations to humans are attempted. With such an appreciation comes the realization that it is also essential to extend mechanistic studies in the NOD mouse to the rat models of spontaneous and induced type 1 diabetes. In addition to the lymphopenic BB/Wor diabetes-prone rat that develops type 1 diabetes spontaneously, type 1 diabetes can be induced in the non-lymphopenic BB/WOR diabetes-resistant substrain and in other strains by immunomodulation coupled with exposure to a parvovirus, Kilham rat virus[5]. Thus, it is the rat and not the mouse model that the investigator should first consider if the object is to an-

alyze the potential of environmental viral pathogens to serve as diabetogenic triggers. New rat models spontaneously developing type 1 diabetes, such as the non-lymphopenic Komeda diabetes-prone (KDP) rat[4], should provide additional pathogenic insights as they become available.

At a minimum, investigations of NOD mice have enhanced our appreciation of the etiologic complexity of type 1 diabetes in humans and provided an example of how promising results obtained in an animal model can be translated into human clinical trials. However, exploitation of the peculiarities of the NOD genome for clinical research is yet to be fully realized. Specifically, further investigation of NOD mice should advance our understanding of the genetic and pathophysiologic basis for other complex pathologies (such as thyroiditis, lupus, sialoadenitis, deafness and inflammatory bowel disease). The strain's robust breeding performance, its extensively characterized genome and the availability of type 1 diabetes-resistant MHC-congenic stocks render NOD mice ideal for outcross with other inbred strains carrying gene mutations for physical mapping/positional cloning analyses. Deficiencies in immunoregulation (such as dysfunctional NK cells or the absence of hemolytic complement) make NOD mice congenic for additional immunodeficiency genes (*scid* and *ray*) ideal hosts for carrying human cells, especially when they are further modified by the transgenic insertion of human genes and/or simultaneous elimination of select murine genes. This technology is providing stocks suitable for analysis of the growth, development and survival of human hematopoietic cells. The immunocompromised NOD mice should also prove useful for the study of human infectious diseases, including AIDS, filariasis and malaria. Gene targeting technology and the ability to produce tissue-specific knockouts of genes are allowing dissection of pathogenic pathways not easily amenable to study in humans.

In sum, criticisms of the inbred nature and controlled housing environment, the ability to change natural physiology through genetic manipulation and the relative ease for disease prevention have caused some to question whether the model is 'as good as it gets'. It is clear that the course of type 1 diabetes development in randomly breeding humans will not be as easily deviated as it is in highly inbred rodent models in which genetic risk is a constant such that interventions can be initiated at very early stages of pathogenesis. Thus, no investigator should assume that the available mouse and rat models spontaneously developing type 1 diabetes represent complete surrogates for humans. However, the fact that diabetes in these rodents develops spontaneously rather than in response to investigator-induced gene targetings allows acquisition of essential insights into the interactions between genes and environment that together trigger a complex disease.

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A NOVEL NOD-DERIVED MURINE MODEL OF PRIMARY SJÖGREN'S SYNDROME

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Objective. **The appearance of autoimmune diabetes prior to autoimmune exocrinopathy in the NOD mouse suggests that it is an excellent model of secondary, but not primary, autoimmune sicca complications. Since the unique major histocompatibility complex (MHC) I-A^{g7} expression in NOD mice is essential for the development of insulitis and diabetes in these animals, we investigated exocrine gland function in NOD.B10.*H2*^{b} mice, which have an MHC congenic to NOD, as a potential model for primary Sjögren's syndrome (SS).**

Methods. **Histopathologic manifestations of lymphocytic infiltrates into the pancreas and exocrine tissues were examined by light microscopy. Sera were evaluated for the presence of antinuclear antibodies. Saliva, tears, and gland lysates were evaluated for total volume and protein concentration, the aberrant expression and processing of parotid secretory protein, and cysteine protease activity.**

Results. **NOD.B10.*H2*^{b} mice exhibited the exocrine gland lymphocytic infiltration typical of the SS-like disease and dysfunction observed in NOD mice, but without the insulitis and diabetes. These mice additionally expressed elevated levels of cysteine protease activity (a measure of apoptotic activity) and abnormal expression and cleavage of parotid secretory protein in the submandibular tissues.**

Conclusion. **The results of this study suggest that the unique NOD MHC I-A^{g7} is not essential for exocrine tissue autoimmunity. Furthermore, the findings indicate that sicca syndrome occurs independently of autoimmune diabetes and that the congenic NOD.B10.*H2*^{b} mouse represents a novel murine model of primary SS.**

Oral and ocular dryness associated with secondary Sjögren's syndrome (SS) is characterized by autoimmune sialadenitis and dacryoadenitis, usually as a complication of another autoimmune disorder (e.g., connective tissue disorders) (1,2). Primary SS is an orphaned autoimmune disorder characterized by the clinical presentation of dryness related to immune destruction that is most obvious in the salivary and lacrimal glands, with occasional satellite involvement of other exocrine tissues (1,2).

Animal models currently used to study secondary SS typically include the lupus-prone MRL/lpr and New Zealand black/New Zealand white mice, carbonic anhydrase–induced experimental autoimmune sialadenitis, and diabetes mellitus–prone NOD mice (3–6). Despite the appearance of lymphocytic foci in the exocrine tissues, only NOD mice develop a corresponding loss of secretory function (6,7). Previously, we reported that NOD mice lose nearly 90% of stimulated saliva and 30% of tear flow between 8 and 20 weeks of age (6–9), the time when the first appearance of lymphocytic foci in the salivary and lacrimal glands occurs. Furthermore, exocrine dysfunction is not dependent on the loss of blood glucose regulation, since male and female nondiabetic NOD mice display this phenotype and since maintenance of diabetic animals on daily insulin supplementation fails to enhance salivary flow (6). In contrast, normal salivary production is observed in NOD-*SCID* mice, indicating that autoimmune lymphocytes are es-

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sential for the development of exocrine complications (9). Therefore, autoimmune exocrinopathy in the NOD murine model of secondary SS most closely resembles the secretory dysfunction observed in human autoimmune primary SS. However, a primary model of spontaneous autoimmune exocrinopathy has yet to be described.

Familial inheritance patterns of Sjögren's syndrome in humans suggest a linkage of disease susceptibility with specific HLA genotypes (1). Similarly, diabetes susceptibility in the NOD mouse is highly dependent on expression of the unique MHC I-A^{g7} and lack of expression of the I-E molecule. Transgenic expression of surface I-E molecules or replacement of the unique I-A^{g7} region with I-A regions from nondiabetic strains results in protection from insulitis and diabetes (10,11). In addition, over 14 independent non-MHC genetic loci may influence the development of diabetes in the NOD mouse (12).

An evaluation of the salivary gland histopathology of various mouse strains has indicated that the I-A^{g7} region is not required for development of lymphocytic infiltration of the salivary glands (10). In contrast, the lack of I-E expression appears to correlate with the age-dependent appearance of lymphocytes in exocrine tissues (11). As a consequence, it could be predicted that MHC congenic partners of NOD expressing an MHC I-A other than g7, yet still lacking I-E expression, would exhibit secretory dysfunction in the absence of insulitis and diabetes, thus establishing a model for primary SS. Following this prediction, the purpose of the present study was to characterize the exocrinopathy in the previously constructed MHC congenic NOD strain, the NOD.B10.$H2^b$ (10).

MATERIALS AND METHODS

Materials. BALB/c, C57Bl/6, B6.NOD.$H2^{g7}$, and NOD/Uf mice (8–12 animals per group) were bred and maintained under specific pathogen–free conditions in the mouse facility at the University of Florida, Gainesville. NOD.B10.$H2^b$ mice were obtained from Dr. Linda Wicker, Merck Research Laboratories (Rahway, NJ), and were bred in the University of Florida animal facility. Both male and female mice at 20 weeks of age were used.

Saliva collection and preparation of gland lysates. Saliva was collected following stimulation of secretion using isoproterenol (0.20 mg/100 gm of body weight) and pilocarpine (0.05 mg/100 gm of body weight) dissolved in saline as described previously (6). Saliva samples were collected for 10 minutes from groups of 8–12 mice on 2 separate occasions and then frozen at −80°C. Excised lacrimal and submandibular glands were homogenized in 10 m*M* Tris buffer (pH 7.4) and immediately frozen at −80°C. Protein assays of both saliva and gland lysates were performed using the method of Bradford (13), with bovine serum albumin as the standard.

Cysteine protease activity. Protease activity in saliva and gland lysates was determined using a standard protease assay as described elsewhere (14,15). The incubation buffer consisted of 25 μl of 100 m*M* BAPNA in DMSO, 10 μl of unknown sample, and 190 μl of phenylmethylsulfonyl fluoride (PMSF) buffer, consisting of 0.2 mg/ml of dithiothreitol, 0.5 mg/ml of disodium EDTA, and 1.0 m*M* PMSF in 100 m*M* phosphate buffer (pH 6.0). Experimental samples, as well as a dilution profile of papain, were incubated at 37°C for 1 hour. The reactions were terminated by the addition of 25 μl of glacial acetic acid, adjusted to 1.0 ml with distilled deionized H_2O, and the optical density was determined at 405 nm to determine the amount of *p*-nitroaniline released. Each assay was performed in duplicate on 3 separate occasions.

Polyacrylamide gel electrophoresis and Western blot analysis. Total salivary proteins (15 μg/well) or gland lysates (50 μg/well) were separated on 10% or 12% sodium dodecyl sulfate–polyacrylamide gels (16). The proteins were transferred to Immobilon-P membranes (Millipore, Bedford, MA) (17) and immunoblotted with a rabbit polyclonal antibody to rat parotid secretory protein (PSP) at a 1:10,000 dilution (18–20). Following 3 10-minute washes, the membranes were incubated with alkaline phosphatase–conjugated goat anti-rabbit immunoglobulin and exposed to substrate as previously described (9). All gels were run on 2 separate occasions for reproducibility of results from individual mice.

Antinuclear antibody (ANA) staining. Sera from the mice (n = 5) were collected by cardiac puncture. Detection of ANA was accomplished with an ANA kit (Sigma, St. Louis, MO) using human hepatocytes (7). A 1:80 dilution of mouse serum was incubated according to the manufacturer's instructions and then incubated with a fluorescein isothiocyanate–conjugated goat anti-mouse second antibody. Nuclear fluorescence was detected by fluorescence microscopy using standard procedures. Nuclear staining was repeated on 3 separate occasions for consistency of observations.

Statistical analysis. All measures of variance are given as standard errors of the mean. Using the Shapiro and Wilk's test, the distributions for saliva and tear volumes, and protein concentration were found to be normal ($P > 0.05$) and were analyzed by a parametric analysis of variance (ANOVA) (21). Tests of ANOVA between independent means were not normal ($P < 0.05$) for cysteine protease activity and were subsequently performed with a nonparametric single-factor test using SAS computer software programs (SAS Institute, Cary, NC). *P* values less than 0.05 were considered significant.

RESULTS

To test the prediction that replacement of the I-A^{g7} locus, while eliminating diabetes, would retain all the biochemical and physiologic characteristics of autoimmune sialadenitis and dacryoadenitis, we studied the NOD.B10.$H2^b$ congenic mouse. To clarify the importance of the MHC, we also examined the converse

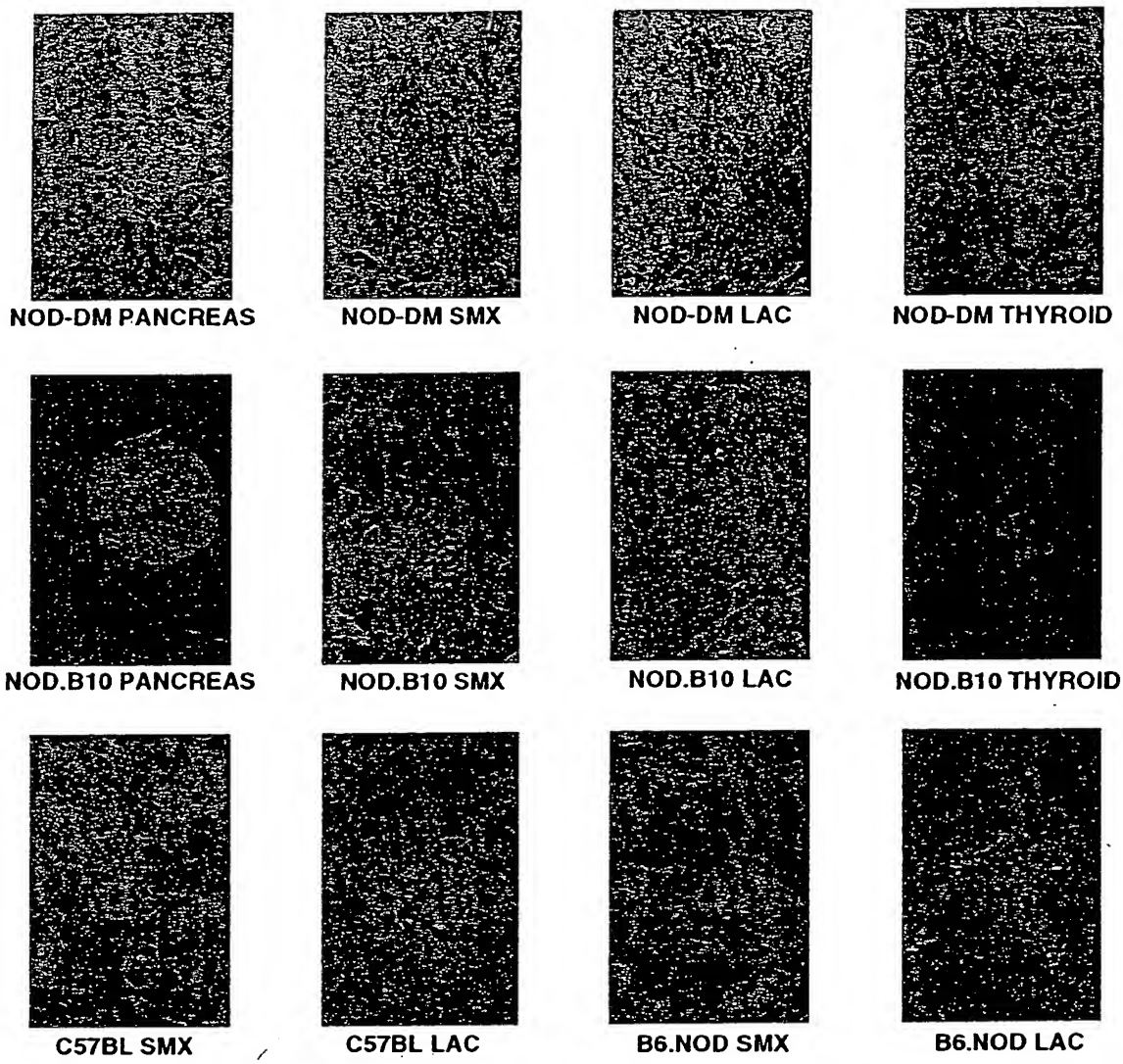

Figure 1. Hematoxylin and eosin–stained histologic sections of the pancreas, submandibular gland (SMX), lacrimal gland (LAC), and thyroid from diabetic NOD (NOD-DM), NOD.B10.$H2^b$, B6.NOD.$H2^{g7}$, and C57Bl/6 mice. Massive perivascular and periductal mononuclear infiltrates are evident in the submandibular, lacrimal, and thyroid gland tissues from the NOD and NOD.B10 major histocompatibility complex (MHC) congenic strains, whereas the NOD MHC in the B6 background or the C57Bl/6 parental strains do not show lymphocytic focal infiltrates. Additionally, there is no evidence of infiltrating cells in the region of the islets of the pancreas of the NOD.B10.$H2^b$ mice. (Magnification × 240.)

B6.NOD.$H2^{g7}$ congenic partner, a strain which contains the C57Bl/6 genetic background with the NOD-derived MHC. NOD and C57Bl/6 mice were used as controls (10).

Submandibular, lacrimal, thyroid, and pancreatic tissues excised from 6-month-old mice were evaluated for the presence of lymphocytic infiltration by histologic staining with hematoxylin and eosin (Figure 1). Only NOD and NOD.B10.$H2^b$ mice developed focal infiltration of the salivary and lacrimal glands, while only the NOD parental strain developed insulitis or diabetes. This is consistent with the findings published by Wicker et al (10) and Yui et al (22) in their initial descriptions of the NOD.B10.$H2^b$ and B6.NOD.$H2^{g7}$ mice, respectively. The presence of leukocytic infiltrates in the exocrine glands of NOD.B10.$H2^b$ mice is striking, in that it shows that the NOD I-A^{g7}, an essential locus for development of

Table 1. Physiologic and biochemical alterations in mice demonstrating Sjögren's syndrome–like exocrinopathy*

Animals	Insulitis/ diabetes	Sialitis/ dacryitis	Saliva volume (μl/10 minutes)	Tear volume (μl/10 minutes)	Saliva protein concentration (μg/μl)	Saliva cysteine protease activity (μg/minute/ml)
NOD (n = 12)	Yes	Yes	106 ± 25†/ 68 ± 15	2.3 ± 0.3‡	6.5 ± 0.5§	43.3 ± 5.8¶
NOD.B10.$H2^b$ (n = 10)	No	Yes	110 ± 27†	1.9 ± 0.4‡	5.2 ± 0.7§	28.2 ± 7.3¶
B6.NOD.$H2^{g7}$ (n = 8)	No	No	233 ± 19	4.6 ± 0.8	3.6 ± 0.3	7.0 ± 2.1
C57Bl/6 (n = 10)	No	No	190 ± 14	4.0 ± 1.1	3.6 ± 0.4	4.5 ± 2.7

* Saliva values for the NOD parental strain were derived from refs. 6 and 7 and are expressed as the volume for prediabetic/diabetic NOD mice. Tear film volume (collected by capillary action) was obtained from 5 mice per group. Values are the mean ± SEM of 3 separate assays performed in duplicate.
† $P < 0.01$ versus B6.NOD.$H2^{g7}$ and versus C57Bl/6 mice.
‡ $P < 0.03$ versus B6.NOD.$H2^{g7}$ and versus C57Bl/6 mice.
§ $P < 0.05$ versus B6.NOD.$H2^{g7}$ and versus C57Bl/6 mice.
¶ $P < 0.03$ versus B6.NOD.$H2^{g7}$ and $P < 0.01$ versus C57Bl/6 mice.

autoimmune diabetes, is not necessary for the development of exocrine gland inflammation. It should be noted that a small percentage of old C57Bl/6 mice, ages 1–2 years, develop spontaneous leukocytic infiltration of the salivary glands (23). These observations suggest that genes contributing to susceptibility to autoimmune sialadenitis might be present in C57Bl/6 mice. However, the addition of the NOD MHC interval to the C57Bl/6 mice did not result in leukocyte infiltration (Figure 1). We did not detect lymphocytic infiltrates in the thyroid sections of either NOD or NOD.B10.$H2^{g7}$ mice, although others have reported this observation (24).

Hallmark traits of autoimmune exocrinopathy in the NOD mouse involve the dramatic loss of secretory function, the aberrant expression and processing of a major saliva protein (PSP), and increased expression of apoptotic cysteine proteases (6,22,25). These physiologic alterations develop in NOD mice between 8 and 20 weeks of age and correlate with the appearance of lymphocytic infiltration of exocrine tissues. To evaluate secretory dysfunction in congenic NOD.B10.$H2^b$ mice, saliva and tear fluid was collected following secretagogue administration.

As shown in Table 1, NOD.B10.$H2^b$ mice generated only half the amount of saliva following stimulation as did C57Bl/6 and B6.NOD.$H2^{g7}$ mice ($P < 0.01$). The saliva volume produced over a 10-minute collection period was similar to that previously reported for the parental NOD animals (6,7). Tear film generated by NOD.B10.$H2^b$ mice was also greatly diminished (Table 1). Tear film collected by capillary action from NOD and NOD.B10.$H2^b$ mice was approximately half that collected from C57Bl/6 and B6.NOD.$H2^{g7}$ mice ($P < 0.03$).

Similar to the findings in NOD mice, an increased protein concentration, based on unit volume of saliva, was noted in NOD.B10.$H2^b$ mice ($P < 0.05$). B6.NOD.$H2^{g7}$ mice produced saliva protein concentrations similar to those of the C57Bl/6 mice.

The cysteine proteases represent a family of enzymes that have been implicated as intracellular signaling components of apoptosis (26). Previous reports have shown a dramatic elevation of cysteine protease activity in the saliva and salivary glands of NOD and NOD-*SCID* mice, which may be indicative of the histologic observation of acinar cell death in these strains. Consistent with the observations of altered enzyme activity in the parental NOD background, the NOD.B10.$H2^b$ mice had a 7- and 4-fold increase in cysteine protease activity compared with C57Bl/6 and B6.NOD.$H2^{g7}$ mice, respectively ($P < 0.01$ and $P < 0.03$).

Salivary gland lysates prepared from these groups of mice reflected the alterations seen in saliva. The submandibular glands from NOD mice with the B10 MHC domain had elevated levels of cysteine protease activity over those of the controls (8.5 ± 1.7 μg versus 2.4 ± 0.9 and 3.1 ± 0.7 μg of protease activity/minute/mg of gland protein for NOD.B10.$H2^b$, B6.NOD.$H2^{g7}$, and C57Bl/6 mice, respectively; $P < 0.02$). The total glandular protein content in NOD.B10.$H2^b$ mice was 2-fold greater than that in C57Bl/6 mice.

Western blot analysis of gland lysates from individual mice indicated that with increasing age, the NOD.B10.$H2^b$ animals (10 of 12) aberrantly expressed and processed PSP, similar to NOD and NOD-*SCID* mice (9,25). As shown in Figure 2, gland lysates from age- and sex-matched C57Bl/6 (0 of 6) and from

B6.NOD.$H2^{g7}$ (0 of 8) mice did not produce PSP. While the precise mechanism of the abnormal expression of PSP in the submandibular gland is not known, the aberrant processing of the protein appears to be due to de novo expression of a proteolytic enzyme in the salivary glands (24). Generation of a smaller PSP isoform in the NOD genetic background appears with the same kinetics as activation of cysteine protease activity and lymphocytic foci in the tissue.

SS patients typically possess ANA (1,2). Previous analyses of NOD sera have indicated the presence of ANA as well as antibodies directed against acinar and ductal cells of both the parotid and submandibular glands (8). Sera from NOD.B10.$H2^b$ and the B6.NOD.$H2^{g7}$ background were therefore evaluated for the presence of ANA. As shown in Figure 3, very pronounced nuclear rim, nuclear cytoplasm, and cytoplasmic staining of hepatocytes was detected in human Hep-G2 cell sections with sera from NOD.B10.$H2^b$ mice but not with sera from B6.NOD.$H2^{g7}$ or BALB/c mice. The nuclear staining did not demonstrate the punctate nuclear staining typically observed in humans with SS (1,2).

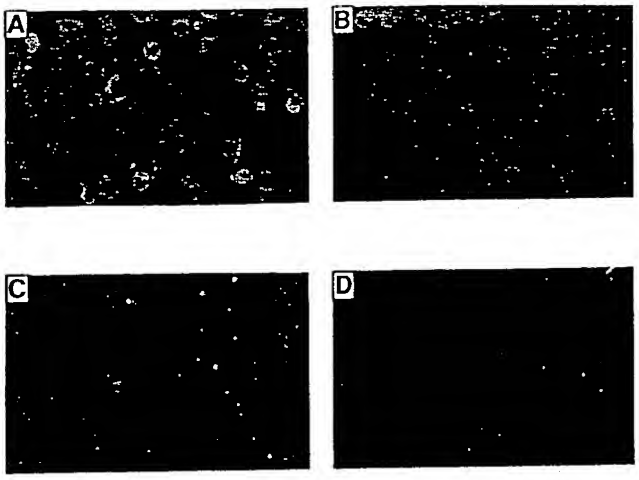

Figure 3. Fluorescence staining of Hep-G2 human hepatocyte nuclei with sera from **A**, diabetic NOD, **B**, NOD.B10.$H2^b$, **C**, B6.NOD.$H2^{g7}$, and **D**, BALB/c mice. Sera were diluted to 1:80 in phosphate buffered saline and incubated with tissue sections according to the manufacturer's instructions (Sigma).

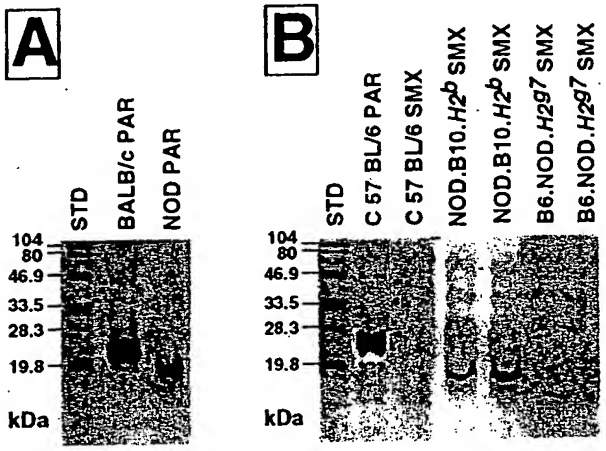

Figure 2. Detection of parotid secretory protein (PSP) in the salivary gland lysates of congenic NOD and control mouse strains. **A**, Western blot detection of PSP in the lysates prepared from the parotid glands of BALB/c and NOD mice, respectively. As shown previously, the NOD mouse produces an aberrantly processed isoform of 17 kd (9). **B**, Representative gland lysates from C57Bl/6, NOD.B10.$H2^b$, and B6.NOD.$H2^{g7}$ submandibular glands (SMX) and parotid glands (PAR). Rabbit polyclonal anti-PSP was a gift from Dr. William Ball (Department of Anatomy, Howard University, Washington, DC). Thirty micrograms of total gland lysates was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (12% gels) followed by transfer to Immobilon-P membrane. Prestained molecular weight standards were as follows: phosphorylase B 104,000 daltons, bovine serum albumin 80,000 daltons, ovalbumin 46,900 daltons, carbonic anhydrase 33,500 daltons, soybean trypsin inhibitor 28,300 daltons, and lysozyme 19,800 daltons. STD = standard.

DISCUSSION

Sjögren's syndrome can be isolated as an autoimmune disease involving primarily the exocrine tissues of the oral cavity and ocular region (primary), or these features in conjunction with the presence of an additional connective tissue disease, such as rheumatoid arthritis or systemic lupus erythematosus (secondary) (1–3). Although the NOD mouse has emerged as the premiere animal model for the study of secondary SS, no naturally occurring model has been identified to date for the study of primary SS (3,6–9). In 1992, Wicker and coworkers (10) demonstrated through histologic analysis that in NOD mice in which the unique I-A^{g7} segment of the MHC region had been replaced by the $H2^b$ haplotype of C57Bl/6 mice, there was a loss of insulitis and diabetes susceptibility. However, these congenic animals retained the histopathologic feature of lymphocytic foci developing in the submandibular gland, reminiscent of SS-like disease. Further biochemical and physiologic analyses presented here show that NOD.B10.$H2^b$ mice exhibit all the pathologic hallmarks (7–9) of the parental NOD mouse model of secondary SS but without the complication of diabetes. This includes the recent observations of increased epithelial cell death, as evidenced by the detection of elevated FAS and Fas ligand staining in

exocrine gland biopsy material from SS patients (27). Thus, the NOD.B10.$H2^b$ mouse represents the identification of the first naturally occurring model for the study of primary SS.

The presence of diabetes in the NOD mouse has previously complicated the development of the NOD mouse model of SS, since insulin and blood glucose levels can influence exocrine gland function. In our experience, the severity of secretory loss in NOD mice increases following the onset of diabetes, which suggests that the loss of insulin secretion plays a role in development of exocrine dysfunction (6). In humans with type 1 insulin-dependent diabetes mellitus, this is reflected by symptoms of dryness in the mouth and eyes in ~33% of patients and appears to be related to the degree of blood glucose control. However, the loss of exocrine gland function in the NOD.B10.$H2^b$ mouse is directly attributable to the autoimmune exocrinopathy.

The observation of underlying pathologic changes in glandular function in the absence of immune components suggests that physiologic changes occur in the NOD background that lead to activation of the immune system (9). Both immune and nonimmune genetic loci appear to contribute to diabetes and SS-like disease in the NOD mouse (12). However, a major chromosomal locus contributing to autoimmune diabetes in the NOD mouse is the unique I-A^{g7} locus, combined with the lack of I-E expression (10–12). The NOD MHC is therefore a major contributing factor for the predisposition to diabetes susceptibility. It has been suggested that susceptibility to autoimmune sialadenitis is, to some extent, dependent on the MHC I-E region (11). Susceptibility to experimental autoimmune sialadenitis in mice in response to the injection of carbonic anhydrase II appears to be restricted to certain *H2* haplotypes (5). Thus, components of the MHC appear to be important contributors to the predisposition to autoimmune diseases.

Detection of autoantibodies directed against exocrine gland surface proteins which are involved in signal transduction that are necessary for the secretory response to occur indicates a prominent role of B lymphocytes and the generation of autoantigens in the process of disease progression that leads to the loss of secretory function (8,28,29). Evaluation of congenic NOD mice, such as the recently bred NOD Igμ^{null} mouse (which lacks B cell maturation and subsequent development of autoimmune diabetes) (30) as well as the NOD.B10.$H2^b$ mouse (which separates autoimmune exocrinopathy from diabetes) will allow us to identify the components required for the development of autoimmune sicca complications.

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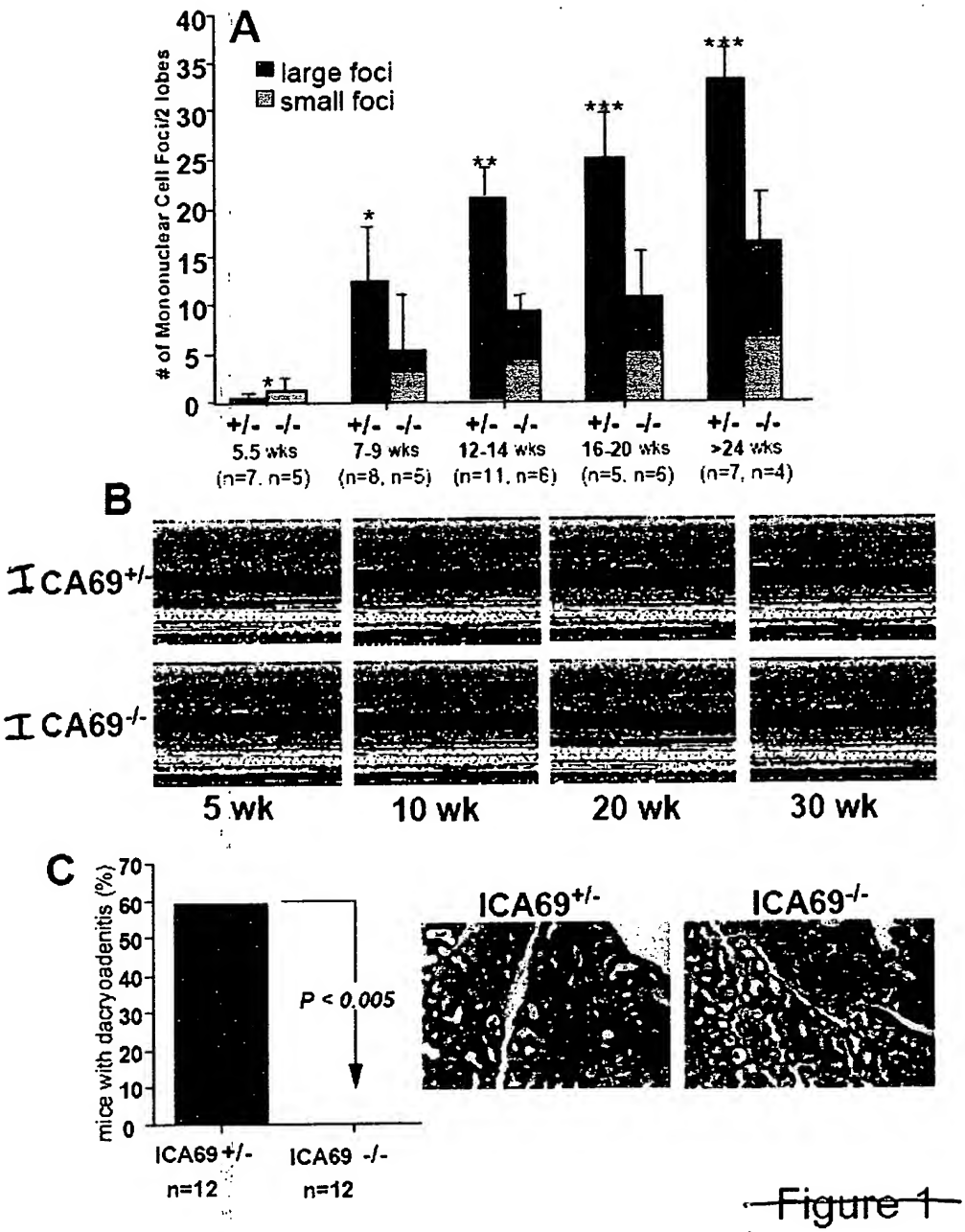
FIGURE 1



~~Figure 1~~

~~2/8~~

FIGURE 2

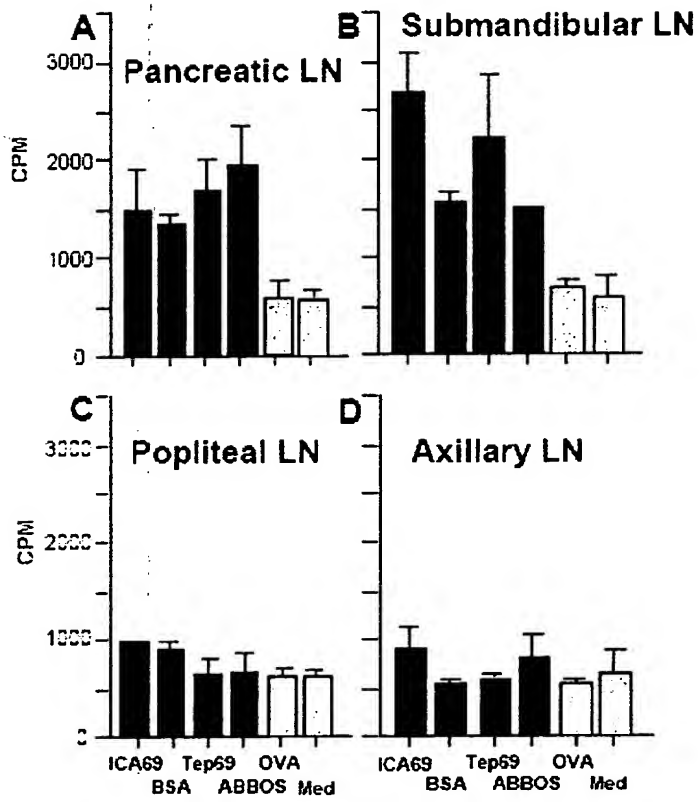


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FIGURE 3

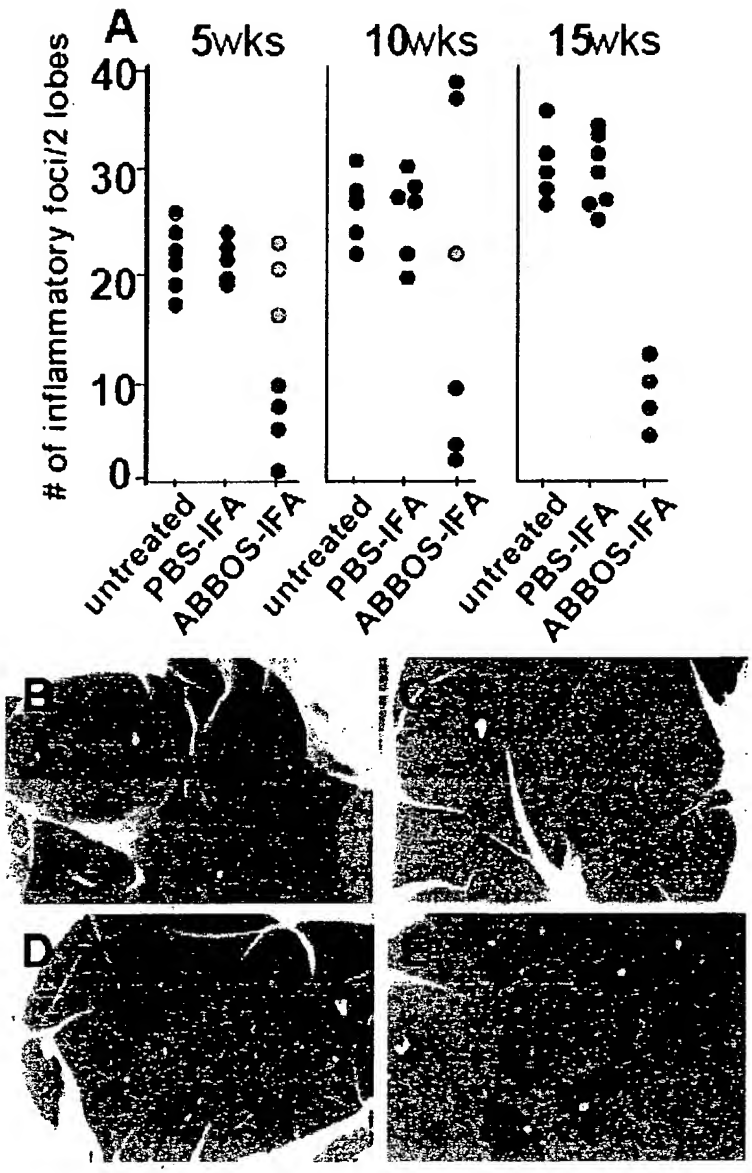

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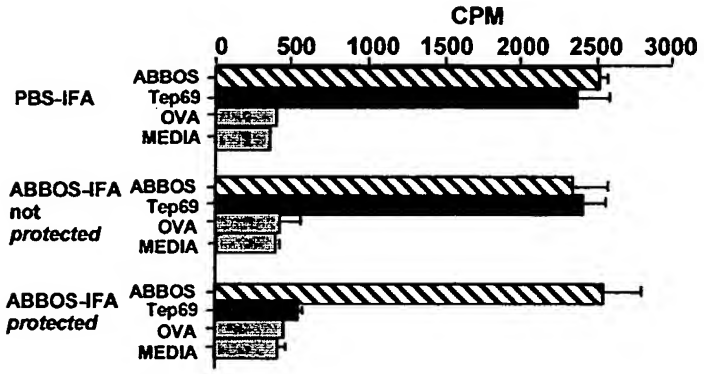
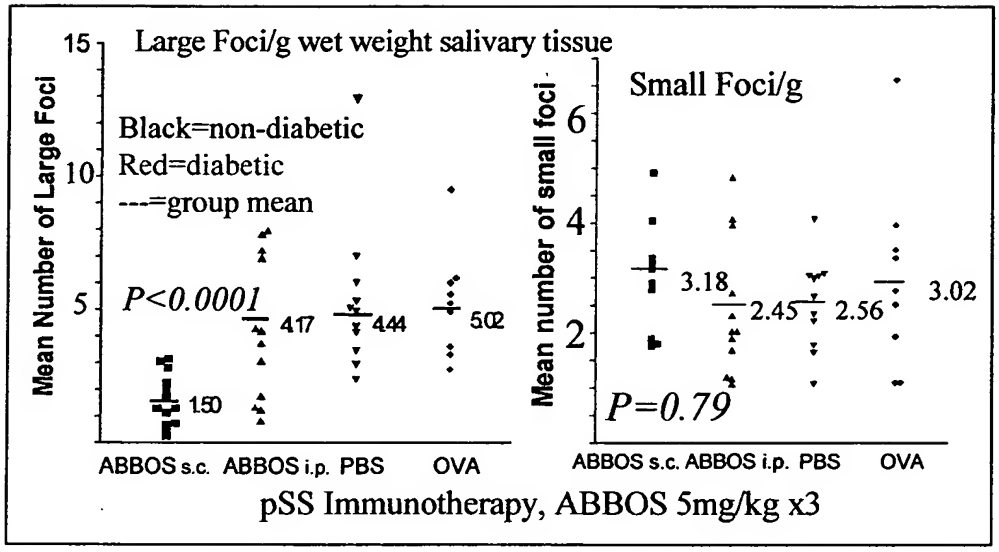
FIGURE 4


CPM
0
500
1000
1500
2000
2500
3000
PBS-IFA
ABBOS
Tep69
OVA
MEDIA
ABBOS-IFA not protected
ABBOS
Tep69
OVA
MEDIA
ABBOS-IFA protected
ABBOS
Tep69
OVA
MEDIA

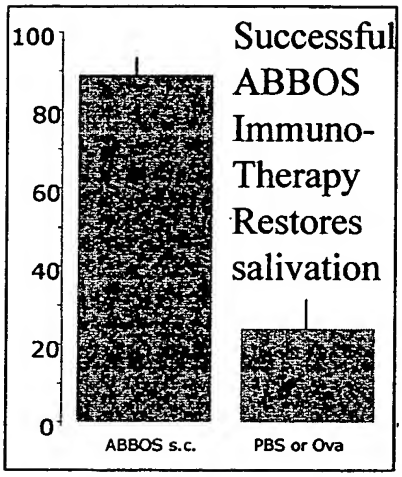
~~5/8~~

FIGURE 5


Large Foci/g wet weight salivary tissue
Mean Number of Large Foci
15
10
5
Black=non-diabetic
Red=diabetic
---=group mean
P<0.0001
1.50
4.17
4.44
5.02
ABBOS s.c.
ABBOS i.p.
PBS
OVA
Small Foci/g
Mean number of small foci
6
4
2
3.18
2.45
2.56
3.02
P=0.79
ABBOS s.c.
ABBOS i.p.
PBS
OVA
pSS Immunotherapy, ABBOS 5mg/kg x3

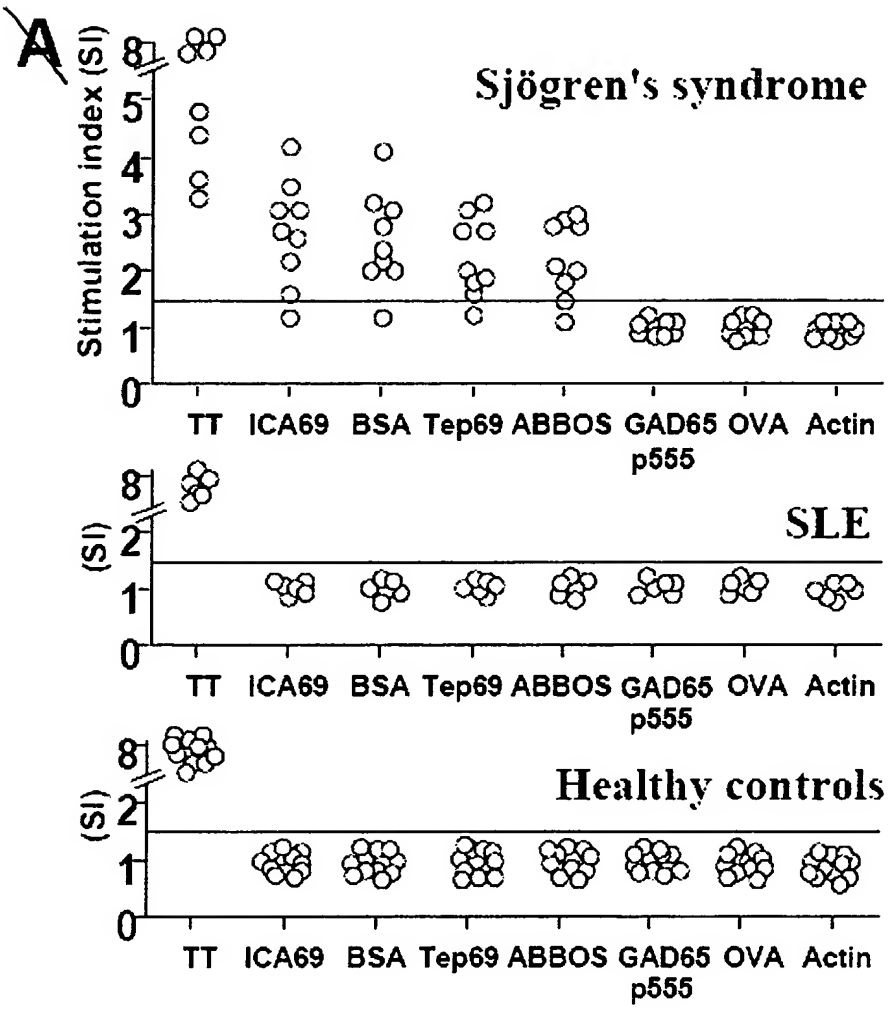
~~6/8~~

FIGURE 6



~~7/8~~

FIGURE 7


A
Stimulation index (SI)
Sjögren's syndrome
SLE
Healthy controls
(SI)
TT ICA69 BSA Tep69 ABBOS GAD65 OVA Actin
p555

~~8/8~~

FIGURE 8